

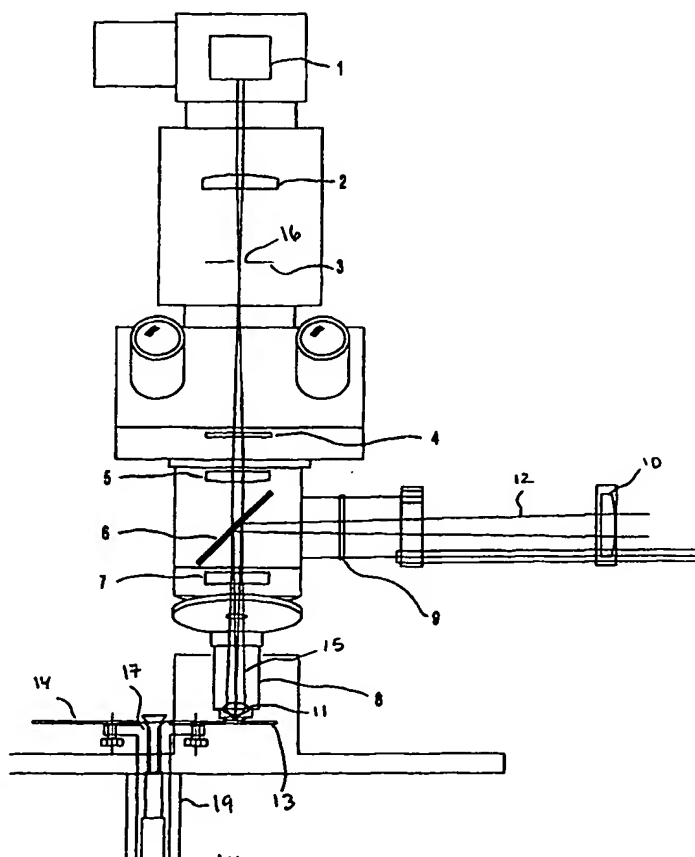


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : G01N 15/14, 35/00, 21/64	A1	(11) International Publication Number: WO 00/20838 (43) International Publication Date: 13 April 2000 (13.04.00)
(21) International Application Number: PCT/DK99/00515 (22) International Filing Date: 30 September 1999 (30.09.99) (30) Priority Data: PA 1998 01243 1 October 1998 (01.10.98) DK (71) Applicant (for all designated States except US): 2C A/S [DK/DK]; Rørmosen 306, DK-2990 Nivå (DK). (72) Inventor; and (75) Inventor/Applicant (for US only): CASPERSEN, Christian [DK/DK]; Rørmosen 306, DK-2990 Nivå (DK). (74) Agent: PLOUGMANN, VINGTOFT & PARTNERS; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).		(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>

(54) Title: AN APPARATUS FOR DETERMINING THE POSITION OF AN OBJECT**(57) Abstract**

The present invention relates to a method and apparatus which can provide rapid scanning of large specimens (14) to detect and determine positions of objects having specific characteristics. The apparatus may, further, be adapted to store information associated with the positions of the detected objects and to retrieve this position information so that an operator may locate a particular type of target object or objects in the specimen for detailed inspection and analysis subsequent to the scanning. The position information may be stored in a volatile or non-volatile memory device provided therefore in a preferred embodiment of the invention. The specimen may be provided on a solid support, such as a circular disc (13) which may be rotatably mounted about an axis (17) of a frame of the apparatus. The objects can be cells or microorganisms of a particular rare type, i.e. they may be present in a very low density in the specimen.

**BEST AVAILABLE COPY**

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

AN APPARATUS FOR DETERMINING THE POSITION OF AN OBJECT

FIELD OF THE INVENTION

5 The present invention relates to a method and an apparatus that provide rapid scanning of large specimens to detect and determine positions of objects having specific characteristics. The apparatus may, further, be adapted to store information associated with the positions of the detected objects and also adapted to identify the objects. The positions of the objects and/or their identities may be stored in a volatile or a non-volatile
10 memory device. The specimen may be provided for scanning on a solid support, such as a circular disc. The objects may be cells or microorganisms of a particular rare type i.e. they may be present in a very low density in the specimen.

BACKGROUND OF THE INVENTION

15

In US 5,374,989 an apparatus for identifying an object having a non-specific outer boundary is disclosed. The apparatus comprises a tracking device for tracking a position of the object to be identified in two dimensions and an identification device for identification of the tracked object. The objects to be identified may be stained so as to
20 distinguish them from other objects by a difference in transmissivity at a specific range of wavelengths. The object identification device comprises a coherent light source illuminating an area of the specimen and a detection device with a plurality of circular coaxial sections which are detecting the intensity distribution of a Fourier transform of light transmitted through the objects of the specimen.

25

US 5,663,057 discloses an apparatus and a process for rapid counting of fluorescent microorganisms in a specimen. A solid support, such as a filter, holding the fluorescent microorganisms is scanned line by line with a laser beam having a circular focus area, with a diameter between 4 and 14 μm , on the filter. The emitted fluorescence is measured
30 at one or more predetermined wavelengths. The scanning lines on the filter are produced in an overlapping pattern, and line-to line features are compared in time synchrony to eliminate uncorrelated events like random noise etc. A digital signal processing circuit applies a set of predetermined mathematical algorithms to the obtained fluorescent light signals to avoid or minimise detection of false positive responses and false negative
35 responses.

The above mentioned types of prior art apparatuses utilise a Cartesian (x,y) scanning and tracking methodology, whereby the specimen is scanned line by line and the particular type of objects are recognised by either detecting emitted light from the objects
5 or by detecting characteristic parameters of an optical Fourier transformation of light transmitted through the objects.

These prior art methods of scanning specimens to locate the objects are too slow for practical purposes when the particular type of objects or target objects are very rare in the
10 specimen, i.e. they may have a density of $1E-6$ or less. The slowly scanning is due to the Cartesian scanning of the specimen and the small focus area of the utilised laser beams combined with the large number of objects, which have to be analysed before it is likely that a target object is located and identified. Further, due to the limited dimensions of the specimen provided by these prior art methods, it may not contain any target objects at all.

15

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method and apparatus which are capable of rapid scanning of large area specimens and, simultaneously,
20 capable of detecting positions of target objects within the specimen.

It is also an object of the present invention to provide an apparatus, which may store the positions of the detected target objects in a volatile or a non-volatile memory device. The positions of the detected target objects may subsequently be retrieved from the memory
25 device, and the objects may be subjected to a thorough analysis in order to establish their identity.

An apparatus according to the present invention may provide a solid support, adapted to receive and hold the specimen, with an area at least twenty times larger than the at most
30 400 mm^2 , utilised in prior art apparatuses.

The apparatus may also be adapted to provide rapid scanning of specimens provided on slides of standard sizes such specimens are commonly used in microscopy in the medical field.

35

The large area of the specimen that may be scanned by the present apparatus is a significant advantage, especially in applications involving detection of very rare target objects, since a substantially larger number of target objects are likely to be present in the specimen than the number of target objects provided by prior art apparatuses.

5

It is further an advantage of the present invention that it may utilise a light source capable of irradiating, on the specimen, a focus area substantially larger than focus areas provided by prior art apparatuses. The large focus area, which may be provided by an embodiment of the present apparatus, enables rapid scanning of even very large specimens.

10

According to a first aspect of the invention there is provided an apparatus for detecting a property of an object contained in a specimen, the apparatus comprising

a frame, a member positioned on the frame and having a surface that is adapted to

15 receive and hold the specimen, a detector for detection of the property of the object, and scanning means for scanning the specimen in relation to the detector along a non-linear curve.

The member may, further, be positioned for rotation about an axis on the frame and the

20 scanning means comprise means for rotating the member about the axis.

The apparatus may further comprise scanning control means for controlling the scanning means to scan the specimen along a predetermined curve, such as a substantially circular curve.

25

According to a preferred embodiment of the invention, the apparatus comprises storage means for storage of a detector signal provided by the detector and one or several corresponding position signals provided by the scanning control means. This embodiment of the apparatus preferably also comprises means for sampling and digitising the detector

30 signal and the corresponding position signal(s).

The apparatus may further comprise signal processing means operationally connected to the detector and adapted to receive and process the detector signal to detect a presence of an object or a particular type of target object or objects. The corresponding position

signal(s) associated with any detected objects may subsequently be stored in the storage means.

The apparatus is preferably adapted to be capable of scanning specimens that have
5 areas larger than 500 mm², or more preferably larger than 2000 mm², or even more preferably larger than 8000 mm².

In one embodiment of the invention, a magnetic property of the target objects is provided for the target objects in the specimen. This can be accomplished by staining the target
10 objects with a magnetic marker. When the specimen is scanned with a magnetic sensitive detector, such as a magnetic reading head, the target object(s) and their positions may be detected by recording an electrical output signal of the reading head and the corresponding position signal(s).

15 In another embodiment of the invention, the target objects are stained with a fluorescent marker and a light source is also provided emitting a first light beam towards the specimen. Further, a light sensitive detector may be provided in this embodiment to detect emitted light from the target object or objects upon interaction with the first light beam during the scanning of the specimen.

20

In embodiments of the apparatus that utilise the light source, the scanning of the specimen may be performed according to anyone of at least three different methodologies. Each of these scan methodologies may correspond to a particular embodiment of the invention, as detailed below.

25

In a first and preferred embodiment of the present apparatus, the member is positioned on the frame of the apparatus. The light source may emit the first light beam towards the specimen, preferably provided on a disc, and the scanning means may be adapted to scan the light beam across the specimen along the non-linear curve. The detector is
30 adapted to detect light emitted from objects comprised in the specimen upon interaction with the first light beam during scanning of the specimen. The member is furthermore positioned for rotation about an axis of the apparatus frame. The scanning means may comprise a DC motor and a spindle rigidly connected to the DC motor. The spindle may, further, be connected to the member which may be a substantially circular disc, holding
35 the specimen to allow the specimen to be rotated about the axis of the apparatus frame.

The scanning means may also comprise deflecting means that may comprise a servo motor or a stepper motor connected to the member holding the specimen and thereby adapted to scan the first light beam along a radius of the circular movement of the disc
5 holding the specimen.

According to another, second, embodiment of the invention, the member holding the specimen is stationary relative to the apparatus frame during the scanning. The light source is positioned for rotation about the axis on the frame and the scanning means may
10 comprise means, which are adapted to rotate the light source about this axis of the frame. The scanning means may, further, comprise deflecting means adapted to scan the first light beam across the specimen along a radius of the circular movement of the light source. This second embodiment is particularly advantageous if the specimen comprising the objects is provided in an unstable e.g. fluid physical state such as in a liquid nutritional
15 solution that allows the target objects to multiply prior to the scanning. This second embodiment of the invention supports scanning of such fluid specimen without the risk of shifting positions of objects in the specimen, since the objects are relieved from centripetal forces produced by rotating the specimen.

20 According to a third and yet other embodiment of the invention, the light source emitting the first beam and the member holding the specimen may be stationary relative to the apparatus frame during the scanning. Instead moveable deflecting means, which may comprise a mirror, may be provided to rotate around a first axis, thereby enabling the first light beam to be scanned across the specimen along a substantially circular curve. The
25 moveable mirror may further be rotatable around a second axis to vary the radius of the circular curve on the specimen so that the specimen may be scanned by the light beam along an e.g. Archimedes' spiral curve.

In the present specification and claims, "a non-linear curve" designates a curve, which
30 can not be generated by any, finite, number of straight lines. Preferably, the non-linear curve is an Archimedes' spiral curve or a curve formed by a number of substantially concentric circular curves.

The target object or objects may be a particular type of biological cell(s), bacteria, micro-
35 organisms, etc. as commonly of relevance in the medical field. In the medical field, these

target cells or target micro-organisms may be found in specimens, which also comprise a large number of resembling cells or micro-organisms. These resembling cells or micro-organisms may constitute the main substance of the specimen, thereby being present in a much larger density than the density of the target cells.

5

The discrimination between the target object(s) and the objects of the main substance may be based on object differences of morphological, magnetic, or optical character.

The target objects are, preferably, stained by utilising antibody coupled magnetic beads or
10 probes conjugated with fluorescent markers to provide them with the required difference from the objects of the main substance, and thereby serve as basis for the discrimination. Both types of antibody probes with fluorescent markers are today commercially available.

The objects may further be provided in a solution and the solution may be deposited on a
15 substantially circular disc that may constitute the solid support adapted to hold the specimen.

An initial enrichment of the solution comprising the objects may be provided before the specimen is submitted for scanning. This initial enrichment may comprise the step of
20 sorting the target objects from the objects of the main substance and/or the step of letting the target objects multiply (through reproduction), thereby enlarging the density of target objects in the specimen prior to scanning.

If a disc is used as the solid support holding the specimen, there exist, in principle, only
25 few limitations to the disc size. Preferably, a disc diameter of approximately 120 mm, which is equal to the diameter of a CD, is utilised. This has several significant advantages, one advantage is that the effective area of a CD size disc is at least twenty times larger than the typical solid support area used in prior art apparatuses. Another advantage is that suitable mechanical and optical parts for the present apparatus may be found readily
30 available and at a very low cost due to the wide spread use of CD based media. The disc may be manufactured in a transparent material, such as polycarbonate, glass, etc.

In a preferred embodiment, the detection of target objects is provided by scanning a first light beam across the specimen along a non-linear curve. This first light beam provides a
35 light spot on the specimen, which irradiates the objects of the main substance together

with any present target objects. The light beam may be provided by a monochromatic coherent light source, such as a semi-conductor laser, gas laser, solid state laser, etc. Alternatively, the light source could be of a type that emits broad-banded light. An optical filter may be inserted in the optical path between the light source and the specimen, so
5 that a substantially monochromatic first light beam is emitted towards the specimen. The use of a broad-banded light source may render expensive laser devices superfluous, and still provide a substantially monochromatic light beam for illumination of the specimen.

The first light beam may comprise light having spectral components at more than a
10 substantially single wavelength. Such a first light beam may be provided by a laser source which is capable of simultaneously generating light components of at least two different wavelengths. Alternatively, a "multi-wavelength" first light beam may be produced by a broad-banded light source combined with a number of optical components inserted in the optical path between the source and the specimen. This last solution may, however, be
15 too complex for practical use.

In a preferred embodiment of the invention, the first light beam is generated by a 488 nm argon-ion gas laser. The target objects in the specimen are, further, stained with at least one fluorescent marker to differentiate them from the objects of the main substance. The
20 specimen is, preferably, dispersed on a disc surface having a diameter of, approximately, 120 mm. The laser beam is focused into a circular spot on the specimen during the scanning. The spot diameter is, preferably, between 20-150 μm , more preferably between 20-80 μm and even more preferably between 30-60 μm . Preferably, the spot diameter is adapted to a particular application so that an optimum signal to noise ratio is provided in
25 the detector signal, thereby enhancing the discrimination between target objects and false positive signals. These false positive signals may originate from random noise in the detector and its associated electronic circuitry, or they may originate from autofluorescent objects and particles, which may have contaminated the specimen.

30 It may be desirable to utilise the largest possible spot diameter in the present apparatus to minimise the scan time of the specimen. Several practical limitations may, however, restrict the maximum possible spot diameter for a particular application. One limitation, in applications involving the detection of fluorescent target objects, may be that a large spot diameter also tends to illuminate a large number of fluorescent contaminated objects and
35 particles. These objects and particles may provide emitted light of intensity larger than the

intensity provided by an illuminated target object, thereby deteriorating the discrimination between target objects and other objects of the specimen or even completely preventing the detection of a target object.

- 5 The maximum light power available from the light source may provide another limitation to the maximum spot size that can be used. However, in a preferred embodiment of the present apparatus, which utilises a laser light source, this potential limitation has not yet been of serious concern.
- 10 Thus, an apparatus according to the present invention may be adapted to identify several types of objects, in a variety of applications, with optimum speed and reliability.

Fluorescent light may be emitted from the target objects during scanning when the first light beam irradiates the target objects and their surroundings. The resultant light,
15 produced by the interaction between the objects of the specimen and the first light beam, may comprise an emitted fluorescent light component from the target objects and a component originating from the first light beam. The resultant light may either be transmitted through the disc surface, reflected from the disc surface, or simultaneously reflected and transmitted. Accordingly, the resultant light may be detected either above
20 the disc surface or below the disc surface when the disc is provided in a transparent material.

The detector is naturally selected so that it is sensitive to the property difference between the target objects and objects of the main substance, this property difference being of
25 morphological, magnetic, optical, etc. character.

In embodiments of the invention where the specimen is scanned by a light source, a light sensitive detector may, obviously, be selected. Several types of devices may be utilised such as CCD devices, photo-transistors, photo-multipliers, etc. depending upon the
30 requirements of a particular application. In a preferred embodiment of the invention, a photo-multiplier is used as a light detector. This type of detector may produce an electrical output signal substantially proportional to the light intensity striking it. The electrical output signal of the detector may, further, be provided to storage means adapted to store the detector signal together with one or several corresponding position signals provided by

the scanning control means, which additionally may be comprised in the present apparatus.

Means may, further, be provided for sampling, digitising and storing one or several
5 electrical signals that may be generated by the at least one detector, such as a photo-multiplier, and the corresponding position signal(s) provided by the scanning control means. Accordingly, each time an object is detected, a corresponding coherent data set may be stored in the storage means, and each of the coherent data sets may be seen as representing a unique signal "event".

10

The coherent data sets may thus represent a number of digitised detector and position signals, the number depending upon the utilised number of detectors and the number of signals provided by the scanning control means. Each of these digitised detector and position signals is, preferably, represented by a series of digital samples generated by
15 one or several A/D-converters. Thereby, each unique signal "event" detected during the scanning of the specimen, and which may or may not originate from a target object, may be represented by a stored coherent data set. Signal "events" not originating from target objects may originate from random noise in the detector and its associated electronic circuitry, or may originate from autofluorescent objects and particles that may have
20 contaminated the specimen as previously explained. These last mentioned signal "events" are denoted false positive signals in the following.

In a preferred embodiment of the invention, a substantially rectangular slit is formed in a (non-transparent) mask inserted in the optical path from the specimen to the photo-
25 multiplier. The photo-multiplier accordingly "sees" the irradiated part of the specimen through a rectangular aperture. The slit or aperture may be placed with its longest side (the length) in parallel with a radius of the circular solid support holding the specimen.

The dimensions of the aperture are, preferably, adapted to the dimensions of the target
30 objects to be detected and located, in such a manner that the width of the aperture is approximately equal to the dimensions of the target objects and the length is approximately equal to 3-5 times the target objects' dimensions, as projected on the specimen. As projected meaning that the dimensions of the aperture should be adapted to any magnification/demagnification provided in the optical path between the specimen and
35 the aperture.

Further, the laser spot irradiating the specimen is, preferably, provided with a diameter approximately equal to the aperture length.

- 5 For example, in an embodiment of the invention wherein the target objects have dimensions of approximately 10 μm , an aperture length of 30-50 μm and an aperture width of between 7.5-15 μm may be selected.

One advantage of applying the aperture in the optical path is that e.g. fluorescent target
10 objects "appearing" within the aperture boundaries at the detector and irradiated by the first light beam, will generate maximum signal level in the detector output signal only during that short instant in time where the target object is fully within the boundaries of the aperture. Consequently, it is possible to detect a very accurate value for the angular co-
ordinate of the target object since the detector signal is not smeared by the target object
15 travelling along a prolonged illumination path.

Another advantage is that the aperture may enhance the achievable discrimination between target objects and objects generating false positive signals due to a difference in size between these object groups. A "large" object, i.e. an object having dimensions larger
20 than the aperture width, and which therefore can not belong to the group of target objects may erroneously have been stained with a fluorescent marker and thus emits light when irradiated by the first light beam. However, such a large object will generate maximum signal strength from the detector during a longer time interval than the target objects, due to a longer time of "visibility" or appearance within the boundaries of the aperture. This
25 difference in signal duration which originates from the size difference between detected objects of the different groups, may be used as a basis for discrimination between target objects and other objects in the specimen, and thus enhance the detection of target objects.

30 Further, the specimen may be scanned, along a radius of the disc holding the specimen, by advancing the position of the aperture and the position of the light spot relative to the specimen with equally large steps i.e. tracking each other. Preferably, the steps have a size of 0.5-1.5 times the dimensions of the target objects. By scanning the first light beam across the specimen according to that method, a pattern of partially overlapping
35 concentric circular curves is produced on the specimen. The overlap of the scan curves

will make a particular target object "appear" at least 2-3 times at the photo-multiplier at a constant rectangular co-ordinate, but at slightly different radial co-ordinates of the aperture. Thereby 2-3 corresponding coherent data sets may be obtained, each set corresponding to electrical signals from the photo-multiplier and the scanning control
5 means.

Signal processing means may subsequently retrieve and use these corresponding coherent data sets to enhance the discrimination between signals originating from target objects and false positive signals. This discrimination may be achieved by analysis of
10 correlation between data sets originating at identical angular co-ordinates but at adjacent radial co-ordinates of the aperture.

When the rectangular aperture is used, an elliptical light spot shape on the specimen may, preferably, cover the entire length of the aperture. The elliptical spot shape may be
15 advantageous since it provides a larger light power within the aperture boundaries than a circular spot, for a fixed light power radiated by the source.

The detection of target objects may be enhanced by inserting one or several optical filters in the light path between the specimen and the detector to attenuate the source light
20 component of the resultant light before it is transmitted to the detector. Preferably, at least one optical filter, preferably a dichroic filter, is inserted in this light path.

The target objects may be stained with different types of fluorescent markers. Preferred marker(s) may be selected according to their optimum excitation light wavelengths and/or
25 their quantum efficiencies. Since, usually, only a particular part of the light spectrum is useful for excitation of a particular type of fluorescent marker, a light source capable of providing light of a sufficiently high intensity relatively close to the optimum excitation wavelength of the marker should be selected.

30 Further, the target objects may be stained with two or more different fluorescent markers so that the target objects may provide emitted light at several wavelengths when they are irradiated by source light of appropriate spectral distribution. This distribution may be provided by the light source, so that it only provides substantial light intensity at wavelengths located close to an excitation wavelength of a marker.

When the target objects are stained with two or more fluorescent markers, target objects in the specimen may be detected by performing successive scans of the specimen, a method hereby denoted "multiple pass scan". The light wavelength of the source may be, during each scan, adapted to excite a particular marker on the objects. The bandwidth of
5 optical filters inserted in the light path between the specimen and the detector may also be adapted to transmit only the wavelength range emitted by the particular fluorescent marker currently being excited by the source light during the scanning.

Alternatively, target objects may be detected in a "single pass scan" of the specimen even
10 though the target objects are provided with e.g. two different markers. This may be achieved by simultaneously exciting both markers with a substantially monochromatic light beam, if the optimum excitation wavelengths of the markers are located sufficiently close in the light spectrum. Light emitted from the target objects may then comprise a light component from each of the markers, and the component originating from each marker
15 may be detected by a separate detector provided for each of the markers. At least one optical filter may be inserted in each optical path from the specimen to the detector. Each filter being adapted to transmit to the detector only light emitted from a particular marker.

Utilising several markers may be advantageous to locate a particular type of target objects
20 possessing several characteristics, and where each characteristic makes the target objects capable of binding a particular type of marker. This type of target objects may be detected and located by, during a first scan of the specimen, locate a first group of objects, which have been stained with a first type of marker, and subsequently during a second scan locate a second group of objects that have been stained with a second type
25 of marker. By determining the intersection of the two groups, e.g. by utilising the signal processing means, the target objects may be identified. Of course, more than two types of markers may be utilised for staining the target objects depending on the particular application and on the number of characteristics of the target objects that have the ability to bind a particular marker.

30

An important application area, illustrating the importance of the binding of different markers to different object characteristics, is provided by the medical field. Detection and analysis of fetal cells from maternal blood provide a very low risk invasive alternative to prenatal diagnostic procedures, such as amniocentesis or chorionic villus sampling. To
35 provide this analysis, at least one fetal cell with a nucleus must be located in a specimen.

The specimen may, further, comprise both maternal cells and fetal cells, and both types may be present with or without a cell nucleus. The detection and location of fetal cells with cell nucleuses may be accomplished by utilising a first fluorescent marker conjugated to a probe, such as an oligonucleotide, which is in situ hybridized to mRNA for gamma globin
5 at least at a part of the fetal cells, and utilise a second fluorescent marker, preferably, DAPI or PI that binds to all cell nucleuses.

By scanning the specimen a first time to locate cells stained with the first fluorescent marker and thus belonging to a first group, and a second time to locate cells stained with
10 the second fluorescent marker, the intersection of the cells groups will contain the target cell(s), i.e. fetal cells that also have a nucleus.

Utilising at least two different markers may also be advantageous to enhance the discrimination between false positive signals and signals originating from target objects
15 during or after the scanning of a specimen. The discrimination may be enhanced in a "multiple pass scan" where the specimen may be scanned in a first pass with light adapted to excite a first marker. Coherent data sets obtained during the scanning, which may correspond to detected objects are stored in the memory. Subsequently the specimen may be scanned a second time with light adapted to excite a second marker
20 and the coherent data sets associated with the detected objects may also be stored in the memory. The discrimination may subsequently be provided by comparing signal values from the detector, obtained at identical angular and radial co-ordinates on the specimen, in the two data sets. One criteria for accepting a detector signal as originating from a target object may be that only signals values, in both data sets, larger than a
25 predetermined threshold value are accepted as originating from a target object. Accordingly, signal values above the threshold in only one of the two data sets may be discarded as originating from noise, autofluorescent particles, etc. and thus being false positive. Identical criteria may, naturally, also be used for discrimination between false positive and "true" detector signals as obtained during a "single pass scan" of the
30 specimen.

A fluorescent marker should, preferably, posses a large quantum efficiency at one hand, and at the other hand posses a large difference between the optimum wavelength of excitation light and the optimum wavelength of emission light.

Preferably, Fluorescein or Fluorescein derivatives may be used as a fluorescent marker(s), since these substances are well known, readily available and possess large quantum efficiencies. Further, optimum wavelengths for excitation light and for emission light are located at 505 nm and 516 nm, respectively, for Fluorescein, which makes it possible to excite this substance by a preferred light source, a 488 nm argon-ion gas laser.

The storage means may be located in a personal computer (PC), which is operationally connected with the apparatus of the present invention. The storage means may comprise magnetic, optic or electric storage media, such as hard disc drives, DAT-tapes, floppy discs, CD-ROM discs, EEPROMs, etc. which may be utilised for non-volatile storage of the coherent data sets obtained from the scanning of the specimen(s). The storage means may also comprise intermediate volatile storage means, preferably RAM, to store coherent data sets during the scanning. This may be advantageous in applications where the data rate provided by the A/D converter(s) is/are higher than the maximum storage rate accommodated by a non-volatile type of memory device.

The storage means may, alternatively, be provided inside the frame of the apparatus in one embodiment of the present invention, thereby providing a convenient portable "stand alone" apparatus.

It some situations, it may be desirable to identify detected target objects after a specimen has been scanned. According to a preferred embodiment of the invention, this is accomplished by retrieving coherent data sets, corresponding to detected target objects, from the memory device. Since these coherent data sets contain information related to the position(s) on the specimen (i.e. may be angular and radial co-ordinates) of the selected target objects, the scanning control means may be adapted to place an automated microscope at the position of any desired target object. Thereby, a medical doctor or a laboratory technician is capable of performing a detailed examination of the target object to e.g. establish its identity.

According to a second aspect of the invention there is provided a method of detecting a property of an object contained in a specimen and comprising the steps of

positioning the specimen on a member having a surface that is adapted to receive and hold the specimen,

scanning the specimen in relation to the detector along a non-linear curve, and

5

detecting the property of the object during scanning of the specimen.

The method may further comprise the step of rotating the member holding the specimen about an axis.

10

According to a preferred embodiment, the method furthermore comprises the step of storing detector signals relating to the detected property and storing corresponding position signals relating to the current position of the member. Preferably, the method further comprises the steps of sampling, digitising and storing these detector and position signals are as coherent data sets in a memory device.

15

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows an embodiment of an apparatus according to the invention including

20 components provided in an optical path.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Fig. 1 shows a preferred embodiment of an apparatus according to the invention, adapted to scan large a specimen 14. A member 17 positioned on a frame (not shown) may hold a substantially circular disc 13 with a diameter of 120 mm, the disc providing a specimen 14, which is submitted for scanning.

25

The specimen may comprise material originating from a blood or tissue sample from a pregnant woman. The specimen may comprise both adult cells and fetal cells, which may be analysed as a part of a prenatal diagnostic procedure. Accordingly, in the present embodiment of the invention, it is highly desirable to detect and determine positions of fetal cells and in particular fetal cells having a cell nucleus so that an analysis of the chromosomes in the cell nucleus may be provided. Accordingly, target cells in the present application are those fetal cells that comprise a cell nucleus.

30

35

However, it is today believed in the medical community that these fetal cells may have a density as small as $1E-9$ - $1E-5$ in the specimen, and they may be present in as little as 50 % of the population of pregnant women. This very low density makes it necessary to
5 perform a rapid scanning of the specimen if the fetal cells are to be located in a reasonable amount of time.

In order to locate the fetal target cells in the specimen, they are, preferably, stained with Fluorescein or Fluorescein derivative markers.

10

Preferably, a coherent first light beam 12, from a 488 nm argon-ion gas laser (not shown) is emitted towards the specimen 14 creating an approximately $40\text{ }\mu\text{m}$ diameter laser spot on the specimen 14. The specimen 14 is scanned by this laser spot along a non-linear curve under the control of scanning means 18 comprising DC motor (not shown) and
15 scanning control means (not shown).

The DC motor, which may be directly connected to the member 17 holding the disc 13 through spindle 19, provides a drive mechanism that enables the disc 13 to be rotated about an axis on the frame. The DC motor further comprises an angle encoder (not
20 shown) capable of providing a signal related to the current angular co-ordinate of the member 17 and the disc 13 to the scanning control means. The angular resolution of the angle encoder is, preferably,

$$\theta_0 = \frac{360}{20000} = 0.018 \text{ Degrees.}$$

25

The rotational speed of the disc 13 is, preferably, adjusted to be within the interval from 200 to 1500 rpm. The scanning control means may comprise servo means adapted control the rpm of the disc, to produce a substantially constant linear velocity of the laser spot on the disc surface, a principle well known from CD players.

30

Deflecting means, which form part of the scanning means, and comprise a stepper motor (not shown), are further provided so the disc 13 with the specimen 14 may be displaced along a radius of the disc and its circular movement. Thereby providing a scan of the entire surface area of the disc 13 with the specimen 14 by the laser spot. When the

deflecting means moves the disc 13 with the specimen along the radius, a non-linear curve comprising a number of concentric partly overlapping circular curves is formed on the disc 13 surface by the laser spot.

- 5 The optical system of the apparatus comprises a 488 nm argon-ion gas laser (not shown) used as a light source. The first light beam 12 emitted from the laser is transmitted through a focusing lens 10 and a dichroic filter 9 to a dichroic beam-splitter 6. This beam-splitter 6 serves two purposes, first to reflect the first light beam 12 towards the specimen 14, and second to filter and direct resultant light 15 emitted from the specimen 14 towards
- 10 a photo-multiplier 1. The resultant light 15 may comprise a light component originating from a reflected portion of the first light beam 12 and a fluorescent light component emitted from fluorescent target objects (not shown) contained in the specimen 14. The dichroic beam-splitter 6 and the dichroic filter 4 both contribute to attenuate the light component originating from the laser source (not shown), thereby enhancing the signal to
- 15 noise ratio of light transmitted to the photo-multiplier 1. The resultant light 15 passing dichroic filter 4 is transmitted through a rectangular slit 16 provided in a mask 3 inserted in the optical path to the photo-multiplier 1. The slit 16 is, preferably, provided with dimensions that result in projected dimensions of length 30 μm and width 15 μm , respectively on the photo-multiplier 1. Accordingly, the slit 16 creates a light path aperture
- 20 of dimensions, which combined with magnification lens 11, defines the dimensions of the irradiated part of the specimen as "seen" by the photo-multiplier i.e. the dimensions of the irradiated specimen area projected upon the photo-multiplier 1.

CLAIMS

1. An apparatus for detecting a property of an object contained in a specimen, comprising
 - 5 a frame,

a member positioned on the frame and having a surface that is adapted to receive and hold the specimen,
 - 10 a detector for detection of the property of the object, and

scanning means for scanning the specimen in relation to the detector along a non-linear curve.
- 15 2. An apparatus according to claim 1, wherein the member is positioned for rotation about an axis on the frame and the scanning means comprise means for rotating the member about the axis.
3. An apparatus according to claim 1 or 2, further comprising scanning control means for
 - 20 controlling the scanning means for scanning the specimen along a predetermined curve.
4. An apparatus according to claim 3, wherein the scanning control means are adapted to control the scanning means in such a way that the predetermined curve is a substantially circular curve.
- 25 5. An apparatus according to claim 3 or 4, further comprising storage means for storage of signals provided by the detector and corresponding position signals provided by the scanning control means.
- 30 6. An apparatus according to claim 5, further comprising means for sampling and digitising the detector signals and the position signals.
7. An apparatus according to any of the preceding claims, further comprising signal processing means operatively connected to the detector to detect a presence of an object
 - 35 based on the detector signals.

8. An apparatus according to claim 7, wherein position signals relating to detected objects are stored in the storage means.
- 5 9. An apparatus according to claim 8, wherein the stored positions of the detected objects are retrieved, and used by said scanning means to position a means for optical inspection of detected objects.
10. An apparatus according to any of the preceding claims, wherein the specimen has an
10 area larger than 500 mm².
11. An apparatus according to any of the preceding claims, wherein the specimen has an area larger than 8000 mm².
- 15 12. An apparatus according to any of the preceding claims, wherein the detector comprises magnetic detection means.
13. An apparatus according to claim 12, wherein the detector comprises a magnetic reading head.
- 20 14. An apparatus according to any of the preceding claims, further comprising

a light source for emission of a first light beam towards the specimen held by the member, and wherein
- 25 the detector comprises an optical detector for detection of light emitted from the object upon interaction with the first light beam during scanning of the specimen.
15. An apparatus according to claim 14, wherein the scanning means further comprise
30 deflecting means for scanning the first light beam across the specimen along a radius of the circular movement of the member.
16. An apparatus according to claim 14, wherein the light source is positioned for rotation about an axis on the frame and the scanning means comprise means for rotating the light
35 source about the axis.

17. An apparatus according to claim 14, wherein the scanning means further comprise deflecting means for scanning the first light beam across the specimen along a radius of the circular movement of the light source.

5

18. An apparatus according to claim 14, wherein the scanning means further comprise movable deflecting means for variable deflection of the first light beam.

19. An apparatus according to claim 18, wherein the movable deflecting means comprise
10 a first mirror that is rotatable around a first axis so that the first light beam can be scanned across the specimen along a substantially circular curve.

20. An apparatus according to claim 19, wherein the first mirror is further rotatable around a second axis for variation of the radius of the circular curve.

15

21. An apparatus according to any of claims 14-20, wherein a mask is inserted in the optical path between the specimen and the detector, and

the mask comprises at least one transparent aperture.

20

22. An apparatus according to claim 21, wherein the aperture shape is a substantially rectangular shape.

23. An apparatus according to claims 21 or 22, wherein at least one dimension of the
25 aperture, as projected on the specimen, is between 0.75 and 2 times the dimensions of objects to be detected.

24. An apparatus according to any of claims 14-23, wherein the objects of the specimen are stained with a fluorescent marker.

30

25. An apparatus according to claim 24, wherein the fluorescent marker is Fluorescein.

26. An apparatus according to any of claims 14-25, wherein the light source is a coherent light source.

35

27. A method of detecting a property of an object contained in a specimen and comprising the steps of:

positioning the specimen on a member having a surface that is adapted to receive and
5 hold the specimen,

scanning the specimen in relation to the detector along a non-linear curve, and

detecting the property of the object during scanning of the specimen.

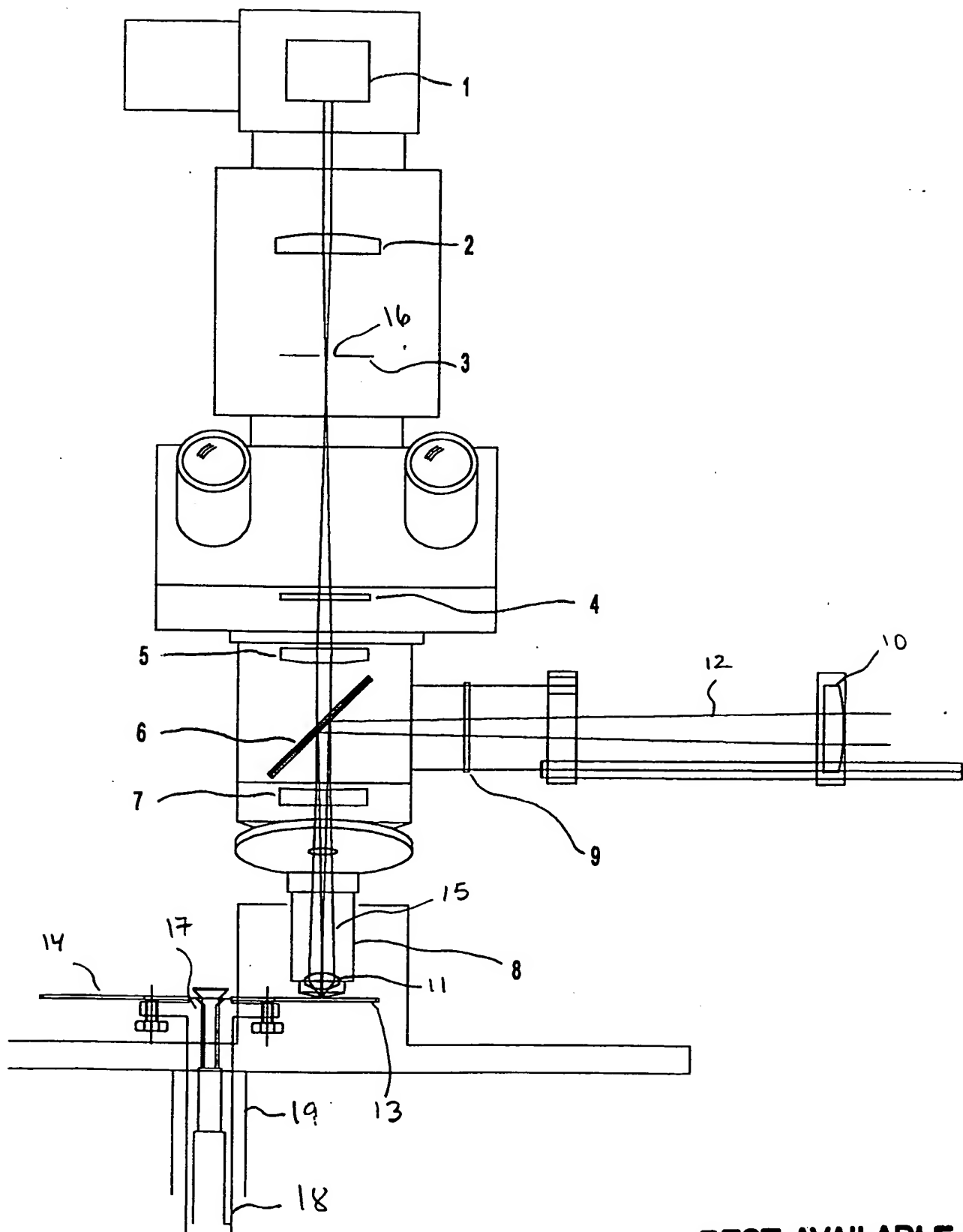
10

28. A method according to claim 27, further comprising the step of rotating the member holding the specimen about an axis.

29. A method according to claim 27 or 28, further comprising the step of storing signals
15 relating to the detected property and corresponding data relating to the current position of the member.

30. A method according to claim 29, further comprising the step of sampling and digitising the signals and the data.

1/1



BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 99/00515

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N15/14 G01N35/00 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP 0 392 475 A (IDEMITSU PETROCHEMICAL CO) 17 October 1990 (1990-10-17) column 5, line 1 - line 38 column 6, line 20 - line 28 column 7, line 37 - line 53 — -/-	1-4, 10, 11, 14, 15, 27, 28 5-8, 12, 13, 16, 18, 21, 22, 24, 26, 29, 30

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

10 December 1999

Date of mailing of the international search report

17/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.

Authorized officer

Marius Montoya E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 99/00515

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>US 4 758 727 A (TOMEI L DAVID ET AL) 19 July 1988 (1988-07-19)</p> <p>column 1, line 38 - line 48 column 2, line 34 - line 38 column 5, line 25 - line 31 column 6, line 19 - line 39 column 7, line 33 - line 35</p>	5-8, 18, 24, 26, 29, 30
Y	<p>US 5 656 429 A (ADELMAN LONNIE W) 12 August 1997 (1997-08-12) column 2, line 27 - line 48</p>	12, 13
Y	<p>GB 2 243 681 A (GALAI LAB LTD) 6 November 1991 (1991-11-06) page 1, line 1 - line 7 page 1, line 25 - page 2, line 4 page 2, line 24 - line 27 page 4, line 10 - line 21 page 5, line 12 - line 18</p>	16
Y	<p>GB 1 388 107 A (SKINNER G K) 19 March 1975 (1975-03-19) page 4, line 86 - line 113 page 7, line 105 - line 117 figure 2</p>	21, 22
X	<p>WO 96 09548 A (GORDON JOHN FRANCIS ;UNIV DUNDEE (GB)) 28 March 1996 (1996-03-28)</p> <p>page 6, line 24 - page 7, line 13 page 20, line 23 - page 21, line 10</p>	1-8, 10, 11, 14, 27-30
X, P	<p>WO 99 07897 A (ERICOMP) 18 February 1999 (1999-02-18) the whole document</p>	1, 12, 13

INTERNATIONAL SEARCH REPORT

information on patent family members

Initial Application No

PCT/DK 99/00515

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0392475 A	17-10-1990	JP 2269938 A CA 2014294 A	05-11-1990 11-10-1990
US 4758727 A	19-07-1988	US 4877966 A	31-10-1989
US 5656429 A	12-08-1997	AU 689964 B AU 3733995 A CA 2201594 A EP 0781346 A JP 10506786 T WO 9610644 A	09-04-1998 26-04-1996 11-04-1996 02-07-1997 07-07-1998 11-04-1996
GB 2243681 A	06-11-1991	NONE	
GB 1388107 A	19-03-1975	NONE	
WO 9609548 A	28-03-1996	AU 3481595 A BR 9509021 A CA 2200562 A CN 1158659 A EP 0782705 A JP 10504397 T US 5892577 A	09-04-1996 30-12-1997 28-03-1996 03-09-1997 09-07-1997 28-04-1998 06-04-1999
WO 9907897 A	18-02-1999	AU 8778898 A	01-03-1999